The genetics of plant morphological evolution

Kristen A Shepard* and Michael D Purugganan†

Considerable progress has been made in identifying genes that are involved in the evolution of plant morphologies. Elements of the ABC model of flower development are conserved throughout angiosperms, and homologous MADS-box genes function in gymnosperm reproduction. Candidate gene and mapping analyses of floral symmetry, sex determination, inflorescence architecture, and compound leaves provide intriguing glimpses into the evolution of morphological adaptations.

Addresses
Department of Genetics, Box 7614, 3513 Gardner Hall, North Carolina State University, Raleigh, North Carolina 27695, USA
*e-mail: kashepar@unity.ncsu.edu
†e-mail: michaelp@unity.ncsu.edu

Current Opinion in Plant Biology 2002, 5:49–55

1369-5266/02/$ – see front matter

Abbreviations

AG	*AGAMOUS*
AP1	*APETALA1*
ATC	*Arabidopsis thaliana CENTRORADIALIS* homolog
cen	*centroradialis*
CET2	*CEN-like gene from tobacco2*
CYC	*CYCLOIDEA*
DICH	*DICHOTOMA*
KNOX	*KNOTTED-like homeobox*
LFY	*LEAFY*
PEAFIM	*PEAFIMBRIATA*
PI	*PISTILLATA*
QTL	quantitative trait loci
SEP1	SEPALLATA1
TFL1	*TERMINAL FLOWER1*
UFO	*UNUSUAL FLORAL ORGANS*

Introduction

Among more than 250 000 extant angiosperm species, taxa are demarcated by an enormous variety of shoot and root architectures; leaf shapes; and flower, fruit and seed forms. The emergence of these morphological differences correlates (albeit imperfectly) with speciation, whereas the ecological consequences of variations in plant form offer clues to the evolution of adaptations. For these and other reasons, understanding the origin of morphological modifications lies close to the heart of evolutionary biology.

The elucidation of developmental genetic pathways that regulate morphogenesis in model species has provided a new foundation for the study of evolutionary diversification. By identifying genes that contribute to species variation, we can analyze the developmental genetic mechanisms of evolution [1], the historical evolutionary forces that have driven diversification [2,3], and the molecular basis for patterns of morphological character transformations among taxa [4]. In the past several years, studies of flower, inflorescence, and leaf development have yielded exciting glimpses of the genetic basis of morphological evolution in angiosperms. In this review, we are able to provide only a whirlwind tour, but several recent articles have provided overviews and conceptual syntheses of other aspects of the evolution of plant morphologies [5••,6–10].

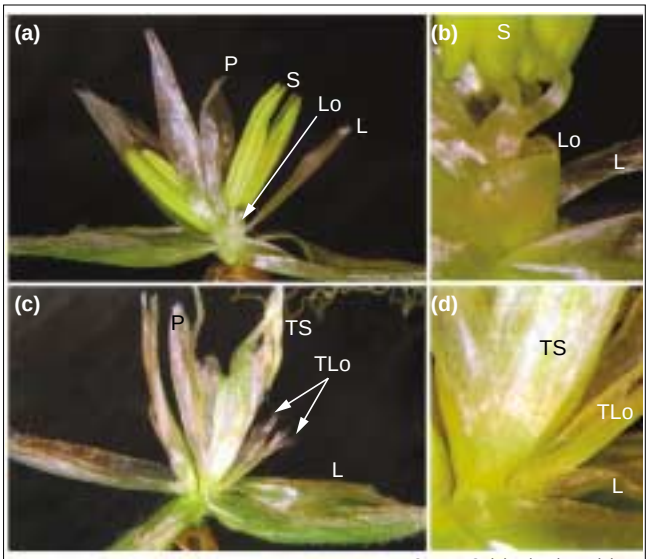
Studying the ABCs (or at least the BCs!) of floral organ identity

Investigations of the molecular evolution of plant development have focused almost exclusively on homeotic genes that are involved in the determination of floral organ identity. According to the well-known ABC model, three classes of transcriptional regulators act in combination to specify sepals (A only), petals (A+B), stamens (B+C), or carpels (C only). In *Arabidopsis thaliana*, A-function is conferred by *APETALA1* (*AP1*) and *AP2*, B-function by *AP3* and *PISTILLATA* (*PI*), and C-function by *AGAMOUS* (*AG*). *AP1*, *AP3*, *PI*, and *AG* are members of the MADS-box gene family; *AP2* belongs to a gene family that is unique to plants (see [11] for review). Recent research has sought to assess the applicability of the ABC model throughout the angiosperms and to understand its relationship to gymnosperm reproductive development.

The specification of petals, stamens, and carpels in *Arabidopsis* requires the additional MADS-box proteins, SEPALLATA1 (SEP1), SEP2, and SEP3 [12•]. Related genes serve similar functions in other eudicots [12•,13]. In *Arabidopsis*, the ectopic expression of *SEP3* and appropriate combinations of homeotic genes convert rosette leaves to petaloid [14•] or staminoid organs [15••]. These results support the hypothesis that all floral organs represent modifications of a leaf-like structure, a central tenet of the ABC model. They also raise the intriguing possibility that *SEP*-like genes play important roles in restricting the activity of floral morphogenetic programs to reproductive organs.

Comparative expression studies indicate that the specification of stamen and carpel identity by B- and C-function genes is conserved throughout angiosperms ([16,17,18••]; see [19] for review), which is consistent with the hypothesis that these organs evolved only once. Analyses of orthologous genes in gnetophytes and conifers suggest that, in the ancestor of seed plants, C-function genes may have specified reproductive development, whereas B-function genes differentiated between male and female reproductive organs (see [9] for review). MADS-box genes have also been isolated from pteridophytes (see [9] for review), lycopods [20], and bryophytes [21], but no unambiguous orthologs of the floral homeotic genes have been found. If more exhaustive sampling fails to uncover orthologs in the basal land plants, it seems reasonable to hypothesize that the diversification of MADS-box genes played a role in the evolution of reproductive morphologies that are associated with heterospory.

Figure 1



Loss-of-function at *Silky1*, a maize homolog of *AP3* and *PI*, results in homeotic transformations of floral organs similar to those observed in B-class mutants of *Arabidopsis*. **(a)** A wildtype tassel spikelet with two male florets. Each floret consists of a palea (P), a lemma (L), three lodicules (Lo), three stamens (S), and an aborted pistil. **(b)** Close up of a wildtype floret. A fleshy lodicule is visible at the base of the stamens. **(c)** A *silky1* tassel spikelet with two florets. The stamens are transformed to pistilloid structures (TS). The transformed lodicules (TLo) resemble the lemma and palea. Note that the transformed stamens do not abort, even though they are composed of pistil-like tissues. **(d)** Close up of *silky1* transformed stamens and transformed lodicule. Photos courtesy of Barbara Ambrose, University of California San Diego. Images reproduced from [22••] with permission.

Analyses of the B-function genes across the angiosperms have stimulated interest in testing two contrasting hypotheses regarding the evolution of petals: first, an ancestral petaloid organ may have arisen early in angiosperm diversification or, second, there may have been multiple origins of petaloid organs. In lower eudicots, *AP3* and *PI* homologs show variations in gene expression that could support a 'separate-origins' hypothesis [17]. Results from studies in grasses (Figure 1; [22••,23]), however, indicate that a petal-specifying program that uses homologs of *AP3* and *PI* could predate the divergence of the monocot and eudicot lineages. In basal dicots, the considerable diversity in the spatial and temporal patterns of B-function gene expression is difficult to reconcile with a single, ancestral petal-specifying program [18••]. Understanding the origin(s) of petals will require additional sampling of taxa throughout the basal angiosperms to clarify whether particular expression variants are typical of larger taxonomic groups or are more recently evolved. Determining when identity-specifying mechanisms became fixed within different lineages may also prove important. Kramer and Irish [18••] speculate that developmental pathways leading to a four-whorled flower, regulated in part by the ABC genes, became canalized in ancestors of the higher eudicot clade. By buffering the effects of mutations on organ-identity specification while permitting the accumulation of genetic variation [24•], this canalization may have facilitated the group's subsequent diversification of flower size and shape [6,18••].

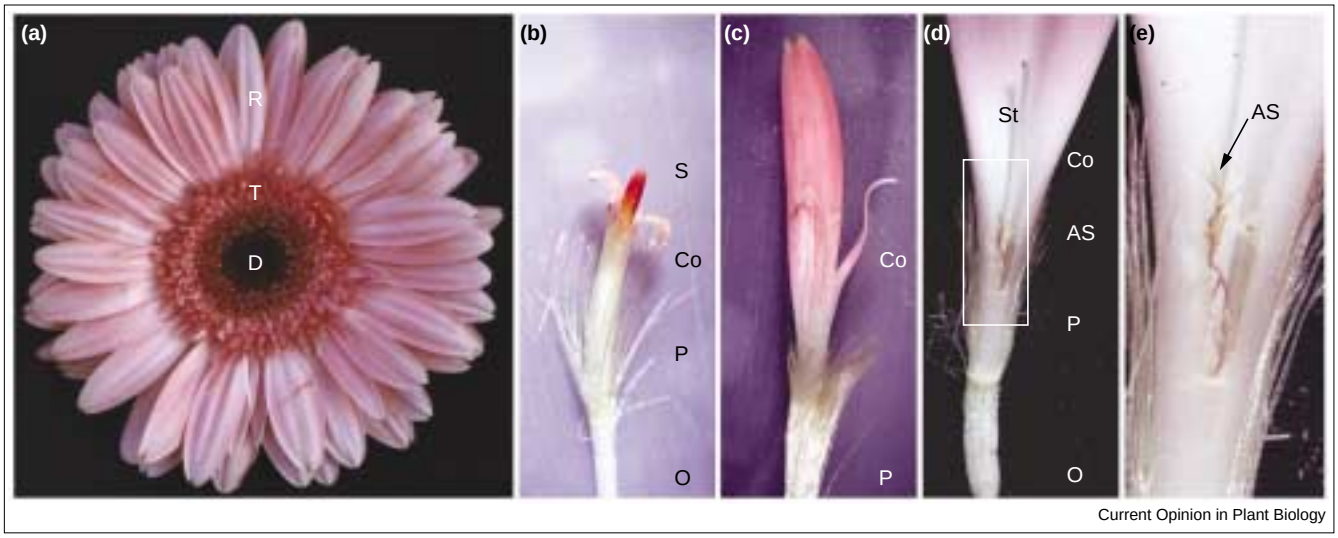
At this point, insufficient data exist to allow an assessment of the evolutionary conservation of perianth-specifying A-function genes. In several monocot and dicot species, putative orthologs of *AP1* are expressed in floral meristems as they are in *Arabidopsis* [23,25,26]. Nevertheless, genes that are clearly related to *AP1* also show divergent expression in plants [16]. To date, *ap1* mutant phenotypes affecting sepal and petal identity have only been observed in *Arabidopsis*. The universality of *AP2* function in sepal and petal specification is even more poorly established. An *AP2* ortholog has recently been characterized in *Petunia hybrida*, but no loss-of-function phenotype was observed for this gene [27]. The identification of an *AP2* ortholog in the gymnosperm *Picea abies* (spruce) may shed light on the ancestral functions of this gene family [28]. Further insight into the evolution of sepals and petals depends on clarification of the roles of A-function genes in the perianths of diverse species.

Molecular phylogenetic studies show that duplication of the floral homeotic genes is quite common. For example, in *Zea mays* (maize), there are three *PI* [29] and two *AG* homologs [30], whereas in *Gerbera hybrida* there are two *AP3* and two *AG* homologs [16]. *AP3* and *PI* duplicates also exist in the lower eudicots and basal angiosperms [17,18••]. Detailed functional analyses of these genes should provide a wealth of information about the importance of gene duplication in plant morphological diversification.

Refinements of floral morphology with adaptive significance

Two common adaptations of floral morphology, unisexuality and zygomorphy (Figure 2), have been studied extensively using developmental genetic approaches. Unisexual flowers, in which stamen or carpel development is selectively repressed, are thought to have evolved independently in many angiosperm lineages (see [31] for review). Several studies fail to support the hypothesis that the evolution of unisexuality involves alterations in the expression patterns of B- or C-function genes ([32]; see [31] for review). Analyses of mutants in maize [22••] and *G. hybrida* [13,16], however, demonstrate that abortion programs affect only organs of the appropriate identity. Thus, in some species, the evolution of unisexuality may require the establishment of new interactions between the floral homeotic genes and pathways involved in growth arrest. Additional mechanisms are also likely as, for example, organ abortion in *Cucumis sativus* (cucumber) is restricted to particular whorls of the flower [33•]. Better understanding of the evolutionary mechanisms involved in sex determination can be achieved by studying clades containing unisexual and bisexual species. A recent phylogenetic study shows that, in some

Figure 2



Gerbera hybrida inflorescences include bisexual and unisexual flowers that also differ in floral symmetry. (a) The gerbera inflorescence contains ray (R), trans (T), and disc (D) florets. (b) Disk florets, which are bisexual, contain fertile stamens (S) and an inferior ovary (O). The lobes of the tubular corolla (Co) are similar in size, resulting in a nearly actinomorphic flower. The style (St) has not yet elongated in this floret. (c) Trans florets are unisexual due to the abortion of anthers relatively late in development. Unequal growth of the corolla lobes yields a zygomorphic flower. The ovary in (c) is outside the field of view, and the style is hidden by the corolla lobes. (d,e) Ray florets produce infertile stamens and strongly zygomorphic corollas. In (d), the two smaller corolla lobes and part of the corolla have been removed to reveal the filamentous remnants of the aborted stamens (AS). The boxed portion is enlarged in (e). (b–d) are shown at the same magnification.

taxa, polyploidization and breakdown of self-incompatibility seem to favor the evolution of sex-determining programs [34], but other selective pressures certainly exist.

Transitions between zygomorphic and actinomorphic flowers have also occurred multiple times in the history of the angiosperms. Zygomorphy (i.e. bilateral symmetry) is important in adaptations to animal pollination. In *Antirrhinum majus* (snapdragon), the putative transcriptional regulators *CYCLOIDEA* (*CYC*) and *DICHOTOMA* (*DICH*) are required in dorsal tissues for zygomorphic development (see [35] for review). Inactivation of a *CYC* ortholog has been implicated in reversion to actinomorphy within a population of *Linaria vulgaris* [36]. In the Gesneriaceae family, however, multiple reversions to actinomorphy do not appear to be associated with changes in the number of expressed *CYC* homologs [37]. Until recently, *CYC* homologs had been studied only in the Lamiales, an ancestrally zygomorphic order. *TCP1* (for *TB1 CYC PCF domain1*), a *CYC* homolog in *Arabidopsis*, is expressed at the adaxial base of floral and axillary meristems [38]. This observation hints that the dorsalizing functions of *CYC* and *DICH* may have evolved from a more general role in branch development.

Studies of the monkeyflowers *Mimulus cardinalis* and *M. lewisii* show that even subtle variations in floral morphology can be ecologically and evolutionarily significant. The flowers of these two species differ in petal lobe shape and orientation, style and anther height, and pigmentation. Although interfertile, these species rarely hybridize naturally. To test the hypothesis that floral morphology contributes to reproductive isolation by encouraging pollinator specificity, Bradshaw and coworkers [39] mapped quantitative trait loci (QTL) for floral variation in *Mimulus* hybrids. For most of the 11 traits studied, several QTL were identified, and at least one QTL explained more than 25% of the total trait phenotypic variance [39]. In field studies of the hybrids, possession of QTL associated with corolla morphology was correlated with the frequency of visitation by bird or bee pollinators [40]. Similar analyses are likely to be useful in understanding intraspecific variation in floral morphology. A recent quantitative genetic study found significant differences in floral traits among *Arabidopsis* ecotypes and recombinant inbred lines (Figure 3), and a mapping experiment identified QTL affecting floral-organ size and shape [41•].

The inflorescence: when and where to make flowers

Variation in inflorescence architecture has long been recognized to have adaptive significance [42]. Among the scores of genes involved in the control of flowering in *Arabidopsis*, *TERMINAL FLOWER1* (*TFL1*) and *LEAFY* (*LFY*) play particularly important roles in inflorescence architecture. TFL1, a putative kinase inhibitor, maintains indeterminacy of inflorescence meristems and influences the number of secondary inflorescences formed. LFY, a transcriptional regulator, promotes floral meristem identity and regulates expression of the floral homeotic genes (see [43] for review). Analyses of *TFL1* and *LFY*

Figure 3

Intraspecific variation in floral morphology in *Arabidopsis thaliana*. Columbia x Landsberg *erecta* recombinant inbred lines show significant quantitative differences in flower mass, organ size, and organ shape. Photo courtesy of Thomas Juenger, University of California Berkeley.

homologs in other angiosperms have begun to address the contribution of these genes to interspecific differences in inflorescence architecture.

The terminal flower phenotype of *centroradialis* (*cen*) mutants of *Antirrhinum* [44] initially suggested that *TFL1* homologs might promote inflorescence indeterminacy in divergent higher eudicots. The absence of expression of similar genes in the determinate inflorescence meristem of *Nicotiana tabacum* (tobacco) appeared to bolster support for a role for *CEN/TFL1* homologs in inflorescence indeterminacy [45]. However, this interpretation has been challenged by a recent phylogenetic analysis showing that *TFL1* and *CEN* are paralogs rather than orthologs [46••]. Furthermore, the members of the *CEN* clade play diverse roles in plant development. The *CEN* clade includes *ATC* (*Arabidopsis thaliana CENTRORADIALIS* homolog) [46••], *SELF-PRUNING* (*SP*) from *Lycopersicon esculentum* (tomato) [47], *CET2* (*CEN-like gene from tobacco2*) and *CET4* [45]. These orthologs exhibit a variety of gene expression patterns, and their loss-of-function phenotypes indicate that *ATC* and *SP* are not involved in inflorescence architecture [45,46••,47]. Functional analyses of additional genes in the *TFL1* clade have not been published, but several apparent orthologs have been isolated: *LpTFL1* from the monocot *Lolium perenne* (perennial ryegrass) [48]; and *CET5* and *CET6* from tobacco [45]. Better understanding of the evolution of inflorescence indeterminacy will require analyses of *TFL1* and *CEN* orthologs in a wider range of species. Because the constitutive expression phenotypes of *TFL1, ATC*, *CEN*, and *LpTFL1* in *Arabidopsis* are similar [46••,48], it is likely that their functional divergence primarily involved changes in gene expression rather than in protein structure.

The expression patterns of *LFY* orthologs vary considerably in the taxa studied so far, and the functional consequences of these differences are still unclear. Shu and coworkers [49•] hypothesize that the expansion of *LFY* expression into the inflorescence meristem accounts for the occurrence of rosette flowering in *Jonopsidium acaule* (violet cress), but a causal relationship has not been demonstrated (Figure 4). The expression pattern of *LFY* homologs in grasses contrasts strongly with its upregulation early in flower development in *Arabidopsis*. *ltLFY* from *Lolium temulentum* (ryegrass) is expressed relatively late in floral development [26], whereas *RFL*, the *FLORICAULA* (*FLO*)–*LFY* homolog of *Oryza sativa* (rice), is expressed in the developing panicle but not in the florets [50]. The apparent diversity of *LFY* expression is surprising given this gene's crucial role in regulating the floral homeotic genes in *Arabidopsis*.

LFY homologs have also been implicated in one of the enduring mysteries of angiosperm evolution: how male and female reproductive organs came to be united in a single structure. Frohlich and Parker [51••] hypothesize in the 'Mostly Male Theory' that the angiosperm flower is derived from a male gymnosperm cone, with the carpel evolving from microsporophylls that produced ectopic ovules. As support for this hypothesis, they note that the single *LFY* locus typical of angiosperm genomes is similar to a *Pinus radiata* gene that is expressed only in male cones. The angiosperm lineage has apparently lost a second *LFY*-like gene that is expressed more broadly in gymnosperms. Frohlich and Parker [51••] predict that other genes active early in floral development should also have homologs expressed in male organs during gymnosperm reproductive development. Although this theory is intriguing, it rests on rudimentary knowledge of the function of either *LFY* homolog in gymnosperms.

The evolution of vegetative morphologies: compound leaf development

Although we've learned a great deal about the evolution of reproductive morphologies, recent genetic studies of vegetative morphological evolution have largely concentrated on mechanisms controlling compound leaf development. It is widely hypothesized that compound leaves evolved independently in many families, but it is also possible that an ancestral developmental program may have been repeatedly suppressed and resurrected in some clades [52]. The evolution of compound leaves in tomato involved expansion of class I *KNOTTED-like homeobox* (*KNOX*) gene activity into leaf primordia. Although *KNOX* gene expression is excluded from leaf primordia in species with simple leaves, tomato leaves express *KNOX* genes during their ontogeny, and overexpression of *KNOX* genes intensifies tomato leaf dissection (see [52] for review). Interestingly, when mutations in *KNOX* repressors permit *KNOX* expression in *Arabidopsis* leaves, lobed leaves develop [53•,54,55]. It is tempting to speculate that the regulation of *KNOX*-mediated effects on leaf margin development provides an easily modified mechanism for generating a variety of degrees of leaf-blade dissection.

Figure 4

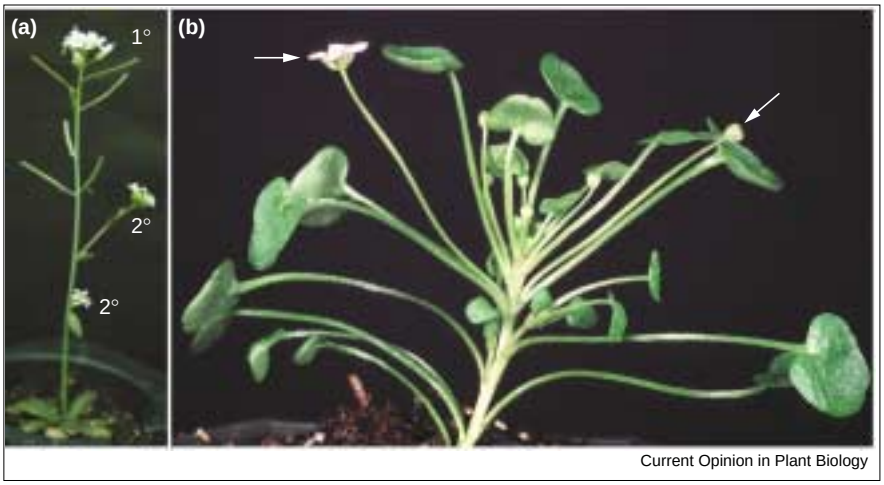


Figure 4. Modification of inflorescence architecture within the Brassicaceae. (a) In *Arabidopsis thaliana*, as in most members of the Brassicaceae, the primary inflorescence (1°) elongates from the basal rosette and bears many flowers that lack subtending bracts. Secondary inflorescences (2°) emerge from the axils of cauline leaves. (b) In striking contrast, *Jonopsidium acaule* produces single flowers (identified by arrows) in the axils of rosette leaves. (b) Photo in courtesy of Darlyne Murawski and David Baum, University of Wisconsin.

Other pathways must also be capable of causing compound leaf development, as pea leaves do not express *KNOX* genes [56]. In pea, *UNIFOLIATA*, a *LFY* ortholog [57•], and *PEA FIMBRIATA* (*PEAFIM*), an ortholog of *Arabidopsis UNUSUAL FLORAL ORGANS* (*UFO*) [58•], are required for compound leaf development and elaboration. Both *LFY* and *UFO* regulate *AP3* expression in *Arabidopsis* flowers via poorly understood interactions [59], and it is possible that elements of a floral development pathway have been co-opted in the evolution of these compound leaves.

Conclusions

Despite a decade of hard work and some truly intriguing results, genetic analyses of plant morphological evolution are still in their infancy. Most studies to date have focused on comparative expression analyses of candidate genes [60•] identified through molecular genetics. Judicious selection of candidate genes requires careful evaluation of a trait's ontogeny and thorough understanding of the candidate gene's mutant phenotypes. Although changes in morphology can certainly be wrought by changes in the spatial or temporal expression of genes, reverse transcription PCR (RT-PCR) and *in situ* hybridization may not reveal the whole story. More exhaustive means of analyzing gene function, such as microarrays, should be informative. Establishing causal relationships between molecules and morphological variants will require comparative studies of closely related yet divergent taxa combined with molecular phylogenetic and population genetic analyses.

Map-based approaches, including QTL mapping, can more directly identify genes responsible for morphological diversification, as was exemplified by Doebley and coworkers with *teosinte branched1* in maize (see [1] for review). Because mapping requires interfertile species, is labor intensive, and can be technically challenging, its utility in interspecific studies may be limited. Intraspecific mapping in *Arabidopsis* and other model plants, however, should allow us to identify new candidate genes for interspecific studies by assigning functions to genes that may not have been uncovered through molecular genetic approaches. More importantly, as morphological diversity between species originates from variation within species, intraspecific studies of natural variation will provide insight into the potential origins of macroevolutionary transformations [61•].

Future genetic studies of plant morphological evolution will (and should) rely on complementary candidate gene and mapping approaches. We hope that the lessons learned from recent studies will soon be applied to a much broader range of questions. A world of biological diversity is waiting to be understood, and leaf, shoot, and root evolution (as well as non-ABC aspects of floral development) are crying out for your attention!

Acknowledgements

We thank members of the Purugganan laboratory for helpful discussions. We are grateful to Barbara Ambrose, David Baum, and Tom Juenger for providing photographs. This work was funded in part by grants to MDP from the National Science Foundation (NSF) and the Alfred P Sloan Foundation.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Carroll SB: Endless forms: the evolution of gene regulation and morphological diversity. *Cell* 2000, 101:577-580.

2. Palopoli MF, Patel NH: Neo-Darwinian developmental evolution: can we bridge the gap between pattern and process? *Curr Opin Genet Dev* 1996, 6:502-508.

3. Purugganan MD: The molecular population genetics of regulatory genes. *Mol Ecol* 2000, 9:1451-1461.

4. Desalle R, Carew E: Phyletic phenocopy and the role of developmental genes in morphological evolution in the Drosophilidae. *J Evol Biol* 1992, 5:363-374.

5. •• Cronk QCB: Plant evolution and development in a post-genomic context. *Nat Rev Genet* 2001, 2:607-619.
The author provides a comprehensive review of key innovations in the evolution of land plants.

6. Endress PK: Origins of flower morphology. *J Exp Zool* 2001, 291:105-115.

7. Endress PK: The flowers in extant basal angiosperms and inferences on ancestral flowers. *Int J Plant Sci* 2001, 162:1111-1140.

8. Lawton-Rauh AL, Alvarez-Buylla ER, Purugganan MD: Molecular evolution of flower development. *Trends Ecol Evol* 2000, 15:144-149.

9. Theißen G, Becker A, Di Rosa A, Kanno A, Kim JT, Munster T, Winter KU, Saedler H: A short history of MADS-box genes in plants. *Plant Mol Biol* 2000, 42:115-149.

10. Vergara-Silva F, Martínez-Castilla L, Alvarez-Buylla ER: MADS-box genes: development and evolution of plant body plans. *J Phycol* 2000, 36:803-812.

11. Jack T: Relearning our ABCs: new twists on an old model. *Trends Plant Sci* 2001, 6:310-316.

12. • Pelaz S, Ditta GS, Baumann E, Wisman E, Yanofsky MF: B and C floral organ identity functions require ***SEPALLATA*** MADS-box genes. *Nature* 2000, 405:200-203.

The authors of this paper show that *sep1 sep2 sep3* triple mutants in *Arabidopsis* produce indeterminate flowers bearing multiple whorls of sepals, even though B- and C-function genes are expressed normally in these mutants. Single *sep* mutants have mild phenotypes. The *SEP* genes act in concert with ABC genes to specify organ identity in the innermost three whorls of the flower.

13. Kotilainen M, Elomaa P, Uimari A, Albert VA, Yu DY, Teeri TH: ***GRCD1***, an ***AGL2***-like MADS box gene, participates in the C function during stamen development in ***Gerbera hybrida***. *Plant Cell* 2000, 12:1893-1902.

14. • Pelaz S, Tapia-Lopez R, Alvarez-Buylla ER, Yanofsky MF: Conversion of leaves into petals in ***Arabidopsis***. *Curr Biol* 2001, 11:182-184.

Rosette leaves are shown to differentiate as petals when they constitutively express *SEP2* or *SEP3* with *AP1*, *AP3*, and *PI*.

15. •• Honma T, Goto K: Complexes of MADS-box proteins are sufficient to convert leaves into floral organs. *Nature* 2001, 409:525-529.

Most leaves and floral organs are shown to differentiate as stamens in *Arabidopsis* plants constitutively expressing *SEP3, AP3, PI,* and *AG*. AP1 is able to substitute for SEP3 in some functions, as co-expression of *AP1*, *AP3,* and *PI* converts leaves to petals. Yeast two-hybrid and *in vitro* assays demonstrate that SEP3 and AP1 proteins form complexes with other ABC proteins. Among the floral homeotic proteins, only SEP3 and AP1 are able to activate transcription *in vitro*. They also appear to have a similar function *in vivo* as shown through analyses of plants bearing reporter constructs.

16. Yu DY, Kotilainen M, Pöllänen E, Mehto M, Elomaa P, Helariutta Y, Albert VA, Teeri TH: Organ identity genes and modified patterns of flower development in ***Gerbera hybrida*** (Asteraceae). *Plant J* 1999, 17:51-62.

17. Kramer EM, Irish VF: Evolution of genetic mechanisms controlling petal development. *Nature* 1999, 399:144-148.

18. •• Kramer EM, Irish VF: Evolution of the petal and stamen developmental programs: evidence from comparative studies of the lower eudicots and basal angiosperms. *Int J Plant Sci* 2000, 161:S29-S40.

A summary of the expression patterns of *AP3* and *PI* homologs in ten species of the lower eudicots and basal angiosperms. Both genes are almost always expressed in stamens, but expression in petaloid organs is variable. Some expression in carpels is also observed. *AP3* and *PI* are not always expressed simultaneously, a surprise as they function as a heterodimer in *Arabidopsis*. The authors include an extensive discussion of hypotheses regarding the evolutionary origin of petals.

19. Ma H, dePamphilis C: The ABCs of floral evolution. *Cell* 2000, 101:5-8.

20. Svensson ME, Johannesson H, Engström P: The ***LAMB1*** gene from the clubmoss, ***Lycopodium annotinum***, is a divergent MADS-box gene, expressed specifically in sporogenic structures. *Gene* 2000, 253:31-43.

21. Krogan NT, Ashton NW: Ancestry of plant MADS-box genes revealed by bryophyte (***Physcomitrella patens***) homologues. *New Phytol* 2000, 147:505-517.

22. •• Ambrose BA, Lerner DR, Ciceri P, Padilla CM, Yanofsky MF, Schmidt RJ: Molecular and genetic analyses of the ***silky1*** gene reveal conservation in floral organ specification between eudicots and monocots. *Mol Cell* 2000, 5:569-579.

Silky1, a homolog of *AP3* and *PI*, is expressed in maize florets, particularly in their stamens and lodicules. In *silky1* mutants, the lodicules are converted to palea-/lemma-like organs and the stamens are replaced by carpeloid organs. These results support the hypothesis that *Silky1* shares functions with B-function genes in eudicots, and suggest that the ABC interactions may predate the divergence of monocots and eudicots. In addition, failure of the homeotic carpeloid structures to abort in whorl 3 of *silky1* female flowers indicates that female sex determination operates specifically on structures with male identity.

23. Kyozuka J, Kobayashi T, Morita M, Shimamoto K: Spatially and temporally regulated expression of rice MADS box genes with similarity to ***Arabidopsis*** class A, B and C genes. *Plant Cell Physiol* 2000, 41:710-718.

24. • Gibson G, Wagner G: Canalization in evolutionary genetics: a stabilizing theory? *Bioessays* 2000, 22:372-380.

The authors discuss the potential importance of canalization in developmental evolution using examples drawn from animal literature.

25. Berbel A, Navarro C, Ferrándiz C, Cañas LA, Madueño F, Beltrán JP: Analysis of ***PEAM4***, the pea ***AP1*** functional homologue, supports a model for ***AP1***-like genes controlling both floral meristem and floral organ identity in different plant species. *Plant J* 2001, 25:441-451.

26. Gocal GFW, King RW, Blundell CA, Schwartz OM, Andersen CH, Weigel D: Evolution of floral meristem identity genes. Analysis of ***Lolium temulentum*** genes related to ***APETALA1*** and ***LEAFY*** of ***Arabidopsis***. *Plant Physiol* 2001, 125:1788-1801.

27. Maes T, Van de Steene N, Zethof J, Karimi M, D'Hauw M, Mares G, Van Montagu M, Gerats T: ***Petunia Ap2***-like genes and their role in flower and seed development. *Plant Cell* 2001, 13:229-244.

28. Vahala T, Oxelman B, von Arnold S: Two ***APETALA2***-like genes of ***Picea abies*** are differentially expressed during development. *J Exp Bot* 2001, 52:1111-1115.

29. Münster T, Wingen LU, Faigl W, Werth S, Saedler H, Theißen G: Characterization of three ***GLOBOSA***-like MADS-box genes from maize: evidence for ancient paralogy in one class of floral homeotic B-function genes of grasses. *Gene* 2001, 262:1-13.

30. Mena M, Ambrose BA, Meeley RB, Briggs SP, Yanofsky MF, Schmidt RJ: Diversification of C-function activity in maize flower development. *Science* 1996, 274:1537-1540.

31. Ainsworth C: Boys and girls come out to play: the molecular biology of dioecious plants. *Ann Botany* 2000, 86:211-221.

32. Brunner AM, Rottmann WH, Sheppard LA, Krutovskii K, DiFazio SP, Leonardi S, Strauss SH: Structure and expression of duplicate ***AGAMOUS*** orthologues in poplar. *Plant Mol Biol* 2000, 44:619-634.

33. • Kater MM, Franken J, Carney KJ, Colombo L, Angenent GC: Sex determination in the monoecious species cucumber is confined to specific floral whorls. *Plant Cell* 2001, 13:481-493.

Expression and genetic studies of *PI* and *AG* orthologs indicate that the arrest of male and female organ development in the cucumber flower depends on position within the flower rather than on organ identity. Petals formed in the third whorl do not abort, suggesting that the sex determination pathway may distinguish between sterile and fertile reproductive organs.

34. Miller JS, Venable DL: Polyploidy and the evolution of gender dimorphism in plants. *Science* 2000, 289:2335-2338.

35. Endress PK: Evolution of floral symmetry. *Curr Opin Plant Biol* 2001, 4:86-91.

36. Cubas P, Vincent C, Coen E: An epigenetic mutation responsible for natural variation in floral symmetry. *Nature* 1999, 401:157-161.

37. Citerne HL, Möller M, Cronk QCB: Diversity of ***cycloidea***-like genes in Gesneriaceae in relation to floral symmetry. *Ann Botany* 2000, 86:167-176.

38. Cubas P, Coen E, Zapater JMM: Ancient asymmetries in the evolution of flowers. *Curr Biol* 2001, 11:1050-1052.

39. Bradshaw HD, Otto KG, Frewen BE, McKay JK, Schemske DW: Quantitative trait loci affecting differences in floral morphology between two species of monkeyflower (***Mimulus***). *Genetics* 1998, 149:367-382.

40. Schemske DW, Bradshaw HD: Pollinator preference and the evolution of floral traits in monkeyflowers (***Mimulus***). *Proc Natl Acad Sci USA* 1999, 96:11910-11915.

41. Juenger T, Purugganan M, Mackay TFC: Quantitative trait loci for
• floral morphology in ***Arabidopsis thaliana***. *Genetics* 2000, 156:1379-1392.
Quantitative genetic analysis revealed significant genetic variation in floral morphology among eight *Arabidopsis* ecotypes and among recombinant inbred lines derived from Landsberg *erecta* and Columbia parents. Eighteen QTL affecting aspects of floral morphology were identified; many of these QTL influenced multiple traits, suggesting either the occurrence of pleiotropy or the presence of tightly linked genes.

42. Tucker SC, Grimes J: The inflorescence: introduction. *Bot Rev* 1999, 65:303-316.

43. Araki T: Transition from vegetative to reproductive phase. *Curr Opin Plant Biol* 2001, 4:63-68.

44. Cremer F, Lönnig WE, Saedler H, Huijser P: The delayed terminal flower phenotype is caused by a conditional mutation in the ***CENTRORADIALIS*** gene of snapdragon. *Plant Physiol* 2001, 126:1031-1041.

45. Amaya I, Ratcliff OJ, Bradley DJ: Expression of ***CENTRORADIALIS*** (***CEN***) and ***CEN-like*** genes in tobacco reveals a conserved mechanism controlling phase change in diverse species. *Plant Cell* 1999, 11:1405-1417.

46. Mimida N, Goto K, Kobayashi Y, Araki T, Ahn JH, Weigel D, Murata M,
•• Motoyoshi F, Sakamoto W: Functional divergence of the ***TFL1***-like gene family in ***Arabidopsis*** revealed by characterization of a novel homologue. *Genes Cells* 2001, 6:327-336.
The *ATC* gene, a *CEN* ortholog and *TFL1* paralog, is expressed in the hypocotyl. An *atc* loss-of-function mutant had no obvious phenotype on its own, and *atc tfl1* double mutants resembled the *tfl1* single mutant. Despite the divergence in their sequences, overexpression of *ATC* and *TFL1* had similar phenotypic consequences. The authors show that the *TFL1* gene family has acquired divergent functions, perhaps mediated by changes in gene expression patterns.

47. Pnueli L, Carmel-Goren L, Hareven D, Gutfinger T, Alvarez J, Ganal M, Zamir D, Lifschitz E: The ***SELF-PRUNING*** gene of tomato regulates vegetative to reproductive switching of sympodial meristems and is the ortholog of ***CEN*** and ***TFL1***. *Development* 1998, 125:1979-1989.

48. Jensen CS, Salchert K, Nielsen KK: A ***TERMINAL FLOWER1***-like gene from perennial ryegrass involved in floral transition and axillary meristem identity. *Plant Physiol* 2001, 125:1517-1528.

49. Shu GP, Amaral W, Hileman LC, Baum DA: ***LEAFY*** and the evolution
• of rosette flowering in violet cress (***Jonopsidium acaule***, Brassicaceae). *Am J Botany* 2000, 87:634-641.
Rosette flowering in violet cress resembles that of *LFY* overexpression mutants in *Arabidopsis.* Violet cress contains two closely related *LFY* homologs, which are strongly expressed in the shoot apical meristem and in flowers. The authors propose that changes in either *LFY* copy number or the regulation of *LFY* expression may have contributed to the evolution of rosette flowering. Violet cress and its relatives represent an excellent clade in which to study the evolution of inflorescence architecture.

50. Kyozuka J, Konishi S, Nemoto K, Izawa T, Shimamoto K: Down-regulation of ***RFL***, the ***FLO***/***LFY*** homolog of rice, accompanied with panicle branch initiation. *Proc Natl Acad Sci USA* 1998, 95:1979-1982.

51. Frohlich MW, Parker DS: The mostly male theory of flower
•• evolutionary origins: from genes to fossils. *Syst Bot* 2000, 25:155-170.
The authors expand on previous studies showing that pairs of *LFY* homologs are present throughout the gymnosperms. Because the single *LFY* gene maintained in the angiosperm lineage is more closely related to a male-specific gene in pine, they propose that flowers are derived from male cones and discuss this hypothesis in the context of the available fossil record.

52. Goliber T, Kessler S, Chen JJ, Bharathan G, Sinha N: Genetic, molecular, and morphological analysis of compound leaf development. *Curr Top Dev Biol* 1999, 43:259-290.

53. Ori N, Eshed Y, Chuck G, Bowman JL, Hake S: Mechanisms that
• control ***knox*** gene expression in the ***Arabidopsis*** shoot. *Development* 2000, 127:5523-5532.
The authors evaluate the expression of *KN1-LIKE from* Arabidopsis thaliana*1* (*KNAT1*)::*GUS* and *KNAT2*::*GUS* reporter constructs in mutants that are affected in leaf margin elaboration. In *asymmetric1* and *asymmetric2* mutants, the *KNAT* genes are ectopically expressed in leaves. When the *asymmetric* loci are crossed into s*errate* or *pickle* mutant backgrounds, leaf lobing and misexpression of *KNAT* are enhanced. These results suggest that repression of *KNAT1* and *KNAT2* gene expression is important for simple leaf development in *Arabidopsis*.

54. Byrne ME, Barley R, Curtis M, Arroyo JM, Dunham M, Hudson A, Martienssen RA: ***Asymmetric leaves1*** mediates leaf patterning and stem cell function in ***Arabidopsis***. *Nature* 2000, 408:967-971.

55. Semiarti E, Ueno Y, Tsukaya H, Iwakawa H, Machida C, Machida Y: The ***asymmetric leaves2*** gene of ***Arabidopsis thaliana*** regulates formation of a symmetric lamina, establishment of venation and repression of meristem-related homeobox genes in leaves. *Development* 2001, 128:1771-1783.

56. Hofer J, Gourlay C, Michael A, Ellis THN: Expression of a class 1 ***knotted 1***-like homeobox gene is down-regulated in pea compound leaf primordia. *Plant Mol Biol* 2001, 45:387-398.

57. Gourlay CW, Hofer JMI, Ellis THN: Pea compound leaf architecture
• is regulated by interactions among the genes ***UNIFOLIATA***, ***COCHLEATA***, ***AFILA***, and ***TENDRIL-LESS***. *Plant Cell* 2000, 12:1279-1294.
The authors show that expression of *UNIFOLIATA*, a *LFY* ortholog, in young leaves is required for compound leaf development in pea. *UNIFOLIATA* is ectopically expressed in several mutants that show greater elaboration of leaf form than is normal.

58. Taylor S, Hofer J, Murfet I: ***Stamina pistilloida***, the pea
• ortholog of ***Fim*** and ***UFO***, is required for normal development of flowers, inflorescences and leaves. *Plant Cell* 2001, 13:31-46.
The authors demonstrate that the classical pea mutant *stamina pistilloida* results from alterations to the *PEAFIM* gene. Although compound leaf development proceeds in the absence of PEAFIM, the number of leaflets per leaf decreases throughout the heteroblastic series. The authors propose that the recruitment of *PEAFIM* into leaf developmental pathways may have permitted the evolution of complex leaf forms.

59. Zhao D, Yu Q, Chen M, Ma H: The ***ASK1*** gene regulates B function gene expression in cooperation with ***UFO*** and ***LEAFY*** in ***Arabidopsis***. *Development* 2001, 128:2735-2746.

60. Haag ES, True JR: Perspective: from mutants to mechanisms?
• Assessing the candidate gene paradigm in evolutionary biology. *Evolution* 2001, 55:1077-1084.
The authors provide an excellent review of the application of candidate gene approaches to the study of plant and animal evolution.

61. Stern DL: Perspective: evolutionary developmental
• biology and the problem of variation. *Evolution* 2000, 54:1079-1091.
The author proposes that combining microevolutionary and developmental studies will reveal much about the nature of mutations that are relevant to macroevolution.